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NOTE ON THE LOCATION OF THE ZEROS OF THE DERIVATIVE OF A RATIONAL FUNCTION HAVING PRESCRIBED SYMMETRY

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The object of this note is to state without detailed proof some new results, representing a sharpening of some known results on the subject of the title.

Jensen's theorem is classical: *If $f(z)$ is a real polynomial, then all non-real zeros of the derivative $f'(z)$ lie on or within the circles (Jensen circles) constructed with diameters the segments joining pairs of conjugate imaginary zeros of $f(z)$.*

For any fixed polynomial $f(z)$ which has non-real zeros, the point set assigned to zeros of $f'(z)$ by Jensen's theorem is unnecessarily large, and can be made smaller by use of Lucas' theorem, that if a convex region K contains the zeros of a polynomial $f(z)$, then K also contains the zeros of the derivative $f'(z)$. In one special case the theorem of Jensen can be considerably improved:

Theorem 1. *Let $f(z)$ be a real polynomial with precisely one pair $(\alpha, \bar{\alpha})$ of conjugate imaginary zeros. Let z_0 be the algebraically least and z_1 the algebraically greatest of the real zeros of $f(z)$. Let A_k $(k = 0, 1)$ be the circular arc bounded by α and $\bar{\alpha}$ of angular measure less than π and tangent to the lines αz_k and $\bar{\alpha} z_k$. Then all non-real zeros of $f'(z)$ lie in the closed lens-shaped region bounded by A_0 and A_1.*

Theorem 1 admits of extension to the case where $f(z)$ has more than one pair of non-real zeros. Here to modify one Jensen circle the rôles of z_0 and z_1 of Theorem 1 may be taken by real zeros of $f(z)$ or by points easily constructed from other Jensen circles. The extended theorem assigns a point set for possible positions of zeros of $f'(z)$ which cannot be improved by use of Lucas' theorem.

Jensen's theorem (like Lucas' theorem) admits extension to the non-Euclidean (hyperbolic) plane:

Theorem 2. *Let $f(z)$ be a real rational function which has no poles interior*

to the unit circle C and whose zeros are inverse to its poles with respect to C. Denote by Jensen circles the non-Euclidean circles constructed using non-Euclidean segments joining pairs of conjugate imaginary zeros of $f(z)$ as non-Euclidean diameters. Then all non-real zeros of $f'(z)$ interior to C lie on or within these Jensen circles.

Theorem 2 may be proved by considering in the extended plane the field of force due to suitable repelling and attracting particles situated at the zeros and poles of $f(z)$; all zeros of $f'(z)$ not zeros of $f(z)$ are positions of equilibrium in this field of force. At an arbitrary point z interior to C but not on Ox nor on or within a Jensen circle, the force has a non-zero component away from Ox in the direction of the non-Euclidean line through z perpendicular to Ox, and this is true for the total resultant force, or for the force due to a pair of particles on Ox situated in a corresponding zero and pole of $f(z)$, or for the force due to four particles situated in a pair of conjugate imaginary zeros of $f(z)$ and their corresponding poles.

Jensen's theorem is a limiting case of and can be proved from Theorem 2.

It may be noted that Theorem 2, generalized by a conformal map, can be expressed as follows:

Let R be a simply connected region which is symmetric in Ox, and let the function $f(z)$ be real on Ox, analytic interior to R, continuous in the corresponding closed region, and of modulus unity on the boundary of R. Then all non-real zeros of $f'(z)$ lie on or within the non-Euclidean circles constructed on the pairs of non-real zeros of $f(z)$ as non-Euclidean diametrically opposite points.

A different type of symmetry leads to different conclusions:

THEOREM 3. *Let $f(z)$ be a rational function whose poles are symmetric to its zeros in the origin O. Let the halves of a double sector with vertex O contain, respectively, all the zeros of $f(z)$ and all the poles of $f(z)$. Then all the zeros of $f'(z)$ also lie in this double sector. If the angle of the double sector is not greater than $\pi/2$, then any circle whose center is O containing all [or no] zeros of $f(z)$ contains all [or no] zeros of $f'(z)$.*

If the angle of the sector here is greater than $\pi/2$, a new circle can be assigned containing all [or no] zeros of $f'(z)$.

There are two categories of results concerning the location of zeros of the derivative of a rational function: (*a*) those which do not depend on the relative multiplicities of the zeros (although symmetry may be prescribed) and (*b*) those which do depend on the multiplicities. Illustrations of (*a*) are Theorems 1, 2 and 3. In the study of the location of the zeros of the derivative $f'(z)$ of a rational function $f(z)$, we ordinarily interpret the zeros of $f'(z)$ which are not also zeros of $f(z)$ as the positions of equilibrium in the field of force due to suitable positive particles situated at the zeros of $f(z)$ and negative particles at the poles of $f(z)$. Whenever situations of type (*a*) are studied, it is useful to separate the zeros and poles of

the given $f(z)$ into groups, chosen as small as possible yet retaining the prescribed symmetry and serving as components in the construction of $f(z)$. For instance in Jensen's theorem a group of zeros of $f(z)$ consists either of a pair of conjugate imaginary zeros or of a single real zero; all poles of $f(z)$ are at infinity. In the general case, for each pair of groups we define the W-curve, namely, the locus of all positions of equilibrium in the field of force due to the two groups of particles where the particles are considered fixed in position with arbitrary (i.e., not necessarily integral) variable multiplicities; but in varying the multiplicities it is not intended that a pole of $f(z)$ should be allowed to replace a zero or reciprocally. Then for the given positions of zeros and poles but with arbitrary multiplicities, *the locus of the zeros of $f'(z)$ is bounded by the W-curves taken for all possible pairs of groups of zeros and poles of $f(z)$.*

In the simplest cases, such as that of Lucas, or the case of a rational function whose zeros are symmetric to its poles with respect to a circle, or the case of a polynomial whose zeros occur in pairs symmetric with respect to a point—in these cases the W-curves and lines of force coincide. In more involved cases such as those of Jensen's theorem or the new results of the present note, the set of lines of force is different from the set of W-curves, and this fact makes proofs more complicated. Nevertheless the W-curves yield important information concerning the zeros of $f'(z)$.

Theorems 1, 2 and 3 can be applied to the study of the location of the critical points of harmonic functions under corresponding conditions of symmetry.

The writer plans to publish elsewhere detailed proofs of the theorems stated and others, with references to the literature.

ON A THEOREM OF GELFAND AND NEUMARK

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Our purpose is to discuss the proof of the following theorem of Gelfand and Neumark:[1]

Let A be a complex Banach space which is at the same time a commutative ring with unit, multiplication satisfying

$$||fg|| \leq ||f||\,||g||,$$

and in which there is defined an automorphism $(^*)$ such that

$$||ff^*|| = ||f||\,||f^*||, \tag{1}$$

$$(fg)^* = f^*g^*,$$
$$(\lambda f + g)^* = \bar{\lambda} f^* + g^*,$$
$$f^{**} = f.$$

Then there exists a compact Hausdorff space Ω such that A is the ring of all continuous complex-valued functions on Ω, and

$$||f|| = \sup_{x \in \Omega} |f(x)|,$$
$$f^*(x) = \overline{f(x)}. \qquad (2)$$

The proof of Gelfand-Neumark uses the simple and elegant technique of normed rings due to Gelfand and others, except in one instance: the hypothesis (1) is used to establish (2) as a result of the existence of a unique minimal closed set $\Omega_0 \subset \Omega$ having the property

$$||f|| = \sup_{x \in \Omega_0} |f(x)|, \text{ for each } f \in A.$$

The authors refer to a rather inaccessible publication for the proof of the existence of this set Ω_0.

We shall give here another way of deducing (2). In order not to repeat obvious portions of reference 1 which do not involve (1) we shall suppose that

$$|f(x)| \leqq ||f||, \text{ for } f \in A \text{ and } x \in \Omega,$$

has been already established, Ω being the space of maximal ideals of A.

Now suppose $f \in A$ and $x \in \Omega$, and

$$f(x) = a + bi,$$
$$f^*(x) = c + di.$$

If $b + d \neq 0$, define

$$g = (f + f^* - au - cu)/(b + d),$$

where u is used to denote the unit element in A.

Then $g = g^*$ and $g(x) = i$. This means $g - iu$ has no inverse in A. Therefore $(g - iu)^* = g + iu$ has no inverse, whence it lies in some maximal ideal y. Thus $(g + iu)(y) = 0$ or $g(y) = -i$.

Since for any real positive N,

$$(g + Niu)(x) = i(1 + N),$$
$$(g - Niu)(y) = -i(1 + N),$$

we have

$$1 + N \leqq ||g + Niu||, ||g - Niu||,$$

$$(1 + N)^2 \leqq ||g + Niu|| \, ||g - Niu||.$$

By (1), the right member

$$= ||g^2 + N^2u|| \leqq N + N^2,$$

where we have now selected $N > ||g^2||$.

The contradiction $(1 + N)^2 \leqq N + N^2$ shows that $b + d = 0$. Applying the same argument to *if* shows that $a = c$, whence $f^*(x) = \overline{f(x)}$.

[1] Gelfand, I., and Neumark, M., "On the Imbedding of Normed Rings into the Ring of Operators in Hilbert Space," *Rec. Math.*, **12** (54), 197–213 (1943).

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*PREVENTION OF ADRENAL CORTICAL CARCINOMA BY DIETHYLSTILBESTROL**, †

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It has been observed that adrenal cortical carcinomas, normally expected in JAX strain ce mice when gonadectomized soon after birth, did not appear following treatment of such mice with a synthetic estrogenic hormone, diethylstilbestrol.

In the present experiments fusion pellets, 25 per cent diethylstilbestrol in cholesterol, av. wt. 4.8 mg., were used.[1] These were introduced into the subcutaneous tissue of the right axilla when the mice were approximately 7 weeks of age. Only one pellet was used at this age in each mouse and none were implanted later in life.

Experimental mice were prepared by removing the gonads of strain ce mice immediately after birth. Adrenal cortical carcinomas had previously been observed in 100 per cent of gonadectomized female mice of this strain 6 months of age and older.[2] In gonadectomized male mice these tumors also had occurred starting at 7 months.[3]

Diethylstilbestrol treated mice of both sexes observed up to 14 months of age did not have these tumors (table 1).

Diet and other environmental factors were kept as near constant as possible in all groups.

Although time has not been sufficient for protection to be observed for mice of extremely advanced ages, the present results are encouraging for continued protection, since nodular hyperplasia of the adrenal cortex which preceded the occurrence of carcinomas in previous experiments was also prevented by the use of diethylstilbestrol pellets.

Further study needs to be made to find a protective agent for tumors of the adrenal cortex which will not at the same time cause other serious body disturbances. In the present experiments pituitary tumors of the type caused by estrogenic hormones were observed in the diethylstilbestrol treated mice as early as 7 months of age.

The problem of whether the presence of pituitary tumors is related to the direct tumor inhibitory action of stilbestrol on the adrenal cortex or whether stilbestrol impairs pituitary action on the adrenal cortex depends for its solution upon further investigation now being planned.

The possibility that the inhibitory action may result from certain effects of a grossly unchanged pituitary must also be considered.

TABLE 1

EFFECT OF DIETHYLSTILBESTROL IN PREVENTING THE OCCURRENCE OF ADRENAL CORTICAL CARCINOMA

MONTHS OF AGE AT AUTOPSY	GONADECTOMIZED FEMALES				GONADECTOMIZED MALES			
	NON-TREATED		STILBESTROL TREATED		NON-TREATED		STILBESTROL TREATED	
	TOTAL NO.	NO. WITH TUMOR	TOTAL NO.	NO. WITH TUMOR	TOTAL NO.	NO. WITH TUMOR	TOTAL NO.	NO. WITH TUMOR
2	2	0	1	0	4	0	1	0
3	3	0	1	0	2	0	1	0
4	4	0	1	0	4	0	1	0
5	1	0	1	0	1	0	1	0
6	5	5	0	0	5	0	0	0
7	4	4	2	0	2	2	2	0
8	1	1	1	0	1	1	1	0
9	2	2	1	0	6	3	1	0
10	2	2	1	0	4	4	0	0
12	6	6	1	0	5	5	1*	0
14	2	2	1	0	1	1	1	0

* This animal was a little under 12 months of age.

The effectiveness of stilbestrol as a substitute for natural gonadal secretion in preventing adrenal cortical tumor formation is, however, clear.

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† Technical assistance of Miss Margaret M. Dickie is gratefully acknowledged.

[1] These pellets were kindly prepared by Dr. Michael B. Shimkin, National Cancer Institute, Bethesda, Md.

[2] Woolley, G. W., and Little, C. C., *Cancer Research*, 5 (4), 193–202 (1945).

[3] Woolley, G. W., and Little, C. C., *Ibid.*, 5 (4), 211–219 (1945).

THE GENETIC CONTROL OF GROWTH METABOLISM*

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In analyzing the effects of genes on development, one basic approach is a determination of the effects of a gene on the metabolic activity of a particular group of cells as reflected by their rate of oxygen consumption. Goldschmidt suggested years ago[1] that genes produce their effects in development by altering the rate of chemical reactions, and more recently the idea that each gene regulates a particular step in a particular biosynthesis has gained favor.[2] In most animals it is impossible to locate exactly the cells which will give rise to a particular structure, but in Drosophila each adult organ develops from a discrete group of cells, an imaginal disc, which can easily be dissected out of the larva. The development of the Cartesian diver ultramicrorespirometer[3] provides a means for determining the oxygen consumption of very small pieces of tissue such as these imaginal discs.

A study was undertaken of the oxygen uptakes of the imaginal discs of wild type and certain mutant stocks of Drosophila. From these one can determine the effect of the substitution of a mutant gene for its wild type allele on the metabolism of the particular group of cells which give rise in the adult to the morphologically altered structure. Thus one more fact can be added to our knowledge of the relation between gene and phenotype. This paper gives the results of a study of the metabolism of wing and leg discs of normal flies and of certain mutant stocks with wings of reduced size. Other experiments are under way to test the effects of the "growth rate" genes, dachs, dachsous, four-jointed, and combgap, and the homoeotic genes, bithorax, tetraltera, and aristapedia, on the metabolism of the imaginal discs involved. It is possible to adapt the Cartesian diver apparatus to test for certain enzymes and coenzymes[4] and in later experiments it may be possible to identify the enzyme system affected by the mutant gene.

Methods.—The stocks of *Drosophila melanogaster* used in the present study were an isogenic wild stock established previously[5] and the mutant stocks "vestigial" (*vg*, chromosome 2, locus 67.0) and "miniature" (*m*, chromosome 1, locus 36.1). Miniature produces wings which are shorter and narrower than normal but of approximately normal shape. Vestigial wings are reduced to small stumps which are held outstretched. Adult miniature wings are about two-thirds normal size and vestigial wings are less than one-quarter normal size.

Two types of imaginal discs were studied, the ventral mesothoracic disc,

which gives rise to the mesothoracic leg and a part of the ventral mesothoracic wall, and the dorsal mesothoracic disc, which becomes the wing and dorsal mesothoracic wall. In the late larva, the dorsal mesothoracic disc has a clearly marked circular region in its posterior part, called the wing bud, from which develops the wing.

Larvae and prepupae were dissected in a drop of phosphate-Locke's solution[6] (pH 7.4), the desired disc was removed and transferred by means of a calibrated braking pipette described by Holter[7] to the diver. The

TABLE 1

RESPIRATION OF IMAGINAL DISCS

DISC	STOCK	AGE	NO. OF DETERMINATIONS	AV. OXYGEN UPTAKE PER DISC PER HR. ± S.E., MM.^{3}/HR.	AV. DRY WEIGHT OF DISC ± S.E., μG.	Q_{O_2}, MM.^{3} O_2/HR./MG.
Wing	Wild	1 hr. after pupation	3	0.028 ± 0.0016	1.35 ± 0.03	20.7
Wing	*m*	1 hr. after pupation	2	0.025 ± 0.0011		18.5
Wing	*vg*	1 hr. after pupation	2	0.013 ± 0.0007		9.2
Wing	Wild	At pupation	2	0.026 ± 0.002	1.32 ± 0.04	19.5
Wing	*m*	At pupation	2	0.024 ± 0.0009		18.1
Wing	*vg*	At pupation	2	0.012 ± 0.0007		8.8
Wing	Wild	2–4 hrs. before pupation	4	0.020 ± 0.0005	0.95 ± 0.03	20.5
Wing	*m*	2–4 hrs. before pupation	4	0.019 ± 0.0005		19.5
Wing	*vg*	2–4 hrs. before pupation	3	0.009 ± 0.0003		9.8
Wing	Wild	5–10 hrs. before pupation	4	0.017 ± 0.0008	0.90 ± 0.04	19.1
Wing	*m*	5–10 hrs. before pupation	4	0.016 ± 0.001		17.3
Wing	*vg*	5–10 hrs. before pupation	5	0.008 ± 0.0013		9.5
Leg	Wild	1 hr. after pupation	4	0.012 ± 0.0017	0.58 ± 0.03	20.1
Leg	*m*	1 hr. after pupation	4	0.013 ± 0.002		22.7
Leg	*vg*	1 hr. after pupation	2	0.012 ± 0.002		20.1
Leg	Wild	2 hrs. before pupation	7	0.0094 ± 0.0006	0.49 ± 0.04	19.1
Leg	*m*	2 hrs. before pupation	3	0.0086 ± 0.0006		17.6
Leg	*vg*	2 hrs. before pupation	4	0.0094 ± 0.001		19.1

divers had a total volume of 8 cu. mm. and contained 1.1 cu. mm. phosphate-Locke's solution in the bulb and 0.9 cu. mm. alkali and 0.9 cu. mm. oil seals in the neck. The "diver constant"[8] of the divers used was 7.8×10^{-4}. All respiration determinations were made at a temperature of 26.8°C.

The quartz fibre balance described by Lowry[9] was used to obtain the weights of the discs. Discs were rinsed several times in glass redistilled

water to remove the phosphate-Locke's solution and transferred to a quartz loop in a drop of distilled water. They were dried in an oven at 100°C. for 30 minutes and then weighed. The hooks were cleaned and reweighed to check the zero point. The balance was calibrated by putting known volumes of standard salt solutions on the hooks, drying and weighing.

Results.—The data of the experiments are given in table 1. At each stage of development the rate of oxygen consumption of the wild type wing discs and of the leg discs of all stocks used varies only slightly from 20 cu. mm. O_2 per hour per milligram of tissue. The Q_{O_2} of the miniature wing discs at each stage of development is slightly less than that of the wild type discs and averages about 18 cu. mm. O_2 per hour per milligram

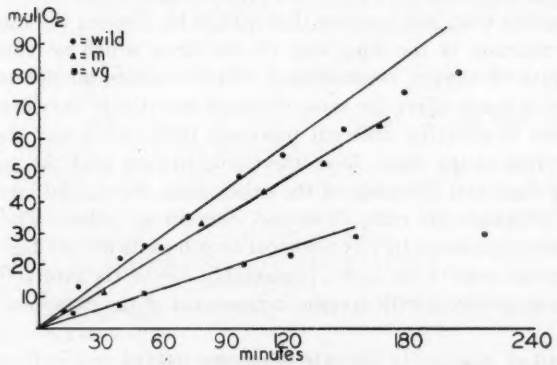

FIGURE 1.

Oxygen uptake in millimicroliters of wild type, vestigial and miniature wing discs dissected out 1 hour after pupation.

of tissue. The differences, though slight, are in the same direction and of about the same magnitude at each stage of development tested and are significant. The Q_{O_2} of the vestigial wing discs is less than half that of the wild type discs and averages about 9 cu. mm. O_2 per hour per milligram of tissue. Both the weight of the wing discs and the rate of oxygen consumption per disc increase sharply in the two-hour period before pupation, but since they increase proportionally, the Q_{O_2} remains constant. Previous embryological studies[10] have shown this period to be one in which a great deal of morphological differentiation occurs.

In all the determinations of oxygen consumption, the experiments were continued from 120 to 240 minutes. The rate of oxygen uptake of the discs was constant for the first 120 minutes or so of each experiment but decreased slightly thereafter (Fig. 1). The discs contain considerable

reserves of substrate and will respire in the divers for twelve hours or more. One wild type wing disc left in a diver overnight consumed so much oxygen that a reading could not be taken, but it had used at least 450 mμl. of oxygen.

Discussion.—The development of the Cartesian diver apparatus and the quartz fibre balance and the peculiarities of the mode of development of Drosophila and other Diptera afford a new approach to the study of gene action. It is possible with this technique to study the effects of particular genes on the rate of metabolism and on the enzyme constitution of the groups of cells which give rise to particular adult organs. Since each gene is believed to function by affecting the nature or quantity of a particular enzyme-controlled biosynthesis, this permits a new attack on the problem of gene physiology. The present study shows that the mutant genes affecting wing size produce their effects by altering the rate of some chemical reaction in the wing disc of the larva which is reflected in a lowered rate of oxygen consumption. These results do not mean that the *m* and *vg* genes affect the same chemical reaction in development, but simply that in affecting different processes they each lower the over-all metabolic rate of the disc. It is interesting to note that the metabolism of the leg discs and probably of the other discs of vestigial larvae is not changed, although the cells, of course, contain *vg* genes. The vestigial gene therefore produces its physiological as well as its morphological effects only in certain cells of the body, presumably due to the interaction of the gene or gene products with specific components of the cytoplasm of those cells.

The marked increase in the rate of oxygen uptake and in the weight of the wing discs which occurs in the two hours before pupation correlates closely with the rapid differentiation of the discs at that time found by morphological studies. The time of pupation is clearly marked by the eversion of the anterior spiracles and the cessation of motion of the larva; the age of the fly relative to this can be determined by inspection. The wing discs are growing more rapidly than the leg discs in the period between two hours before and one hour after pupation. The wing discs show a 43 per cent increase in oxygen uptake and a 42 per cent increase in weight in this period and the leg discs have only a 24 per cent increase in oxygen uptake and an 18 per cent increase in weight. This may be correlated with the morphological finding[11] that the wing buds are more advanced than the leg buds at pupation and have invaginated into the body cavity.

The weights of the wing discs of wild, miniature and vestigial flies taken at corresponding ages were very similar and showed the same range of variation, so they were averaged together (table 1). This finding corroborates Auerbach's statement[12] that *vg* wing discs are the same size as wild

type discs but show less differentiation at corresponding ages. Chen[13] had previously stated that vestigial discs were smaller than wild discs of corresponding ages.

Summary.—The rate of oxygen uptake per gram dry weight of the wing and leg discs of wild type, "miniature" and "vestigial" Drosophila was determined by the Cartesian diver ultramicrorespirometer. Miniature produces a slight and vestigial a marked decrease in the oxygen consumption of the wing disc but neither one affects the respiration of the leg discs. The mutant genes therefore produce their physiological as well as their morphological effects only in certain cells of the body, presumably by an interaction of the genes or gene products with specific cytoplasmic constituents of those cells.

* I am indebted to Drs. E. G. Ball and C. B. Anfinsen for their helpful suggestions and advice in the course of this work, and to C. Lloyd Claff for the use of the Cartesian diver apparatus.

[1] Goldschmidt, R., *Die quantitativen Grundlagen von Vererbung und Artbildung*, I. Springer, Berlin, 1920.

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[4] Anfinsen, C. B., *Jour. Biol. Chem.*, **152**, 285–291 (1944).

[5] Villee, C. A., *Genetics*, **31**, 428–437 (1946).

[6] 1.497 g. KH_2PO_4, 6.248 g. Na_2HPO_4, 4.092 g. NaCl, 0.373 g. KCl, and 0.147 g. $CaCl_2 \cdot 2H_2O$ per liter H_2O.

[7] Holter, H., *C. r. Lab. Carlsberg, Sér. chim.*, **24**, 399–478 (1943).

[8] Linderstrøm-Lang, K., *Ibid.*, **24**, 333–398 (1943).

[9] Lowry, O., *Jour. Biol. Chem.*, **140**, 183–189 (1941).

[10] Chen, T. Y., *Jour. Morph.*, **47**, 135–199 (1929); Robertson, C. W., *Ibid.*, **59**, 351–400 (1936); Auerbach, C., *Trans. Roy. Soc. Edinburgh*, **58**, 787–815 (1936).

[11] Auerbach, C., loc. cit.

[12] Auerbach, C., loc. cit.

[13] Chen, T. Y., loc. cit.

LEAFHOPPER TRANSMISSION OF CORN STUNT*

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A new corn disease prevalent in the Rio Grande Valley of Texas and the San Joaquin Valley of California has been under study since June, 1945, when affected plants were first received from Dr. A. A. Dunlap of the Texas Agricultural Experiment Station, College Station, Texas. The disease caused yellows-type symptoms and resembled, superficially, yellow stripe of corn, transmitted by the leafhopper *Peregrinus maidis* (Ashm.).[1] However, the conspicuous cell inclusion bodies associated with that disease were not found in the plants from Texas and California.

The disease was described from California by Frazier[2] under the name "streak" in March of last year. Altstatt,[3] a little later in the season, described it from Texas. Since the name "streak" already had been applied to another disease of corn, transmitted by the leafhopper *Cicadulina mbila* Naude,[4] the writer designated the new disease as "stunt" in reference to the shortening of internodes and the general stunting it caused. From the descriptions of Frazier and Altstatt and from observations on symptoms, it was concluded that stunt had not been described previously. It also was concluded that Frazier and Altstatt probably were correct in suggesting that it might be a virus disease.

Efforts to transmit stunt by the rubbing method with juice from diseased plants were unsuccessful. Also, attempts to transmit it by dodder, *Cuscuta campestris* Yuncker, failed. Frazier stated that high populations of the leafhopper *Baldulus maidis* (De L. and W.) were frequently found in fields where the disease occurred. Hence an effort was made to transmit stunt by this insect. A virus-free colony, obtained by taking newly hatched nymphs from a diseased corn plant before they had an opportunity to feed, was maintained on healthy corn plants and used in all tests reported below. The tests were carried out with potted plants in large, wood and screen cages and in lantern globe cages in greenhouses.

In one transmission experiment 14 healthy sweet corn plants of the variety Golden Bantam were exposed for 4 days to approximately 150 adult leafhoppers that had been hatched and reared on a diseased corn plant. Fourteen other corn plants of the same age and variety were exposed to about the same number of virus-free adult leafhoppers for 4 days. All of the plants exposed to the first colony came down with stunt in from 5 to 6 weeks after exposure. All of the plants exposed to the control colony remained healthy during a three-month period that they were kept under observation.

In another experiment 7 of 16 New Jersey No. 2 hybrid field corn plants, exposed for one day to a colony of 200 adult leafhoppers that had been hatched and reared on a diseased plant, came down with stunt in from 34 to 38 days after exposure. All of 16 similar plants of the same hybrid exposed to 200 virus-free leafhoppers for one day remained healthy during a period of 85 days that they were under observation.

In still another experiment 15 virus-free leafhoppers were allowed to feed on a diseased corn plant for 5 days and were then transferred to 26 healthy New Jersey No. 2 hybrid field corn plants. Fifteen other virus-free leafhoppers were allowed to feed on a healthy corn plant for 5 days and were then transferred to 26 other healthy corn plants of the same hybrid. After feeding on the two sets of plants for 30 days both groups of insects were removed and destroyed. In from 28 to 40 days thereafter 15 of the 26 plants exposed to the insects that had fed on the diseased corn plant, came down with stunt. The other 11 plants and the 26 control plants remained healthy during a period of 60 days that they were kept under observation.

These experiments and other similar experiments have proved that corn stunt is readily transmitted by *Baldulus maidis*.

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*SOME PROPERTIES OF A GENETIC CYTOPLASMIC FACTOR IN PARAMECIUM**

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Recent work by Sonneborn,[1, 2, 3] Lindegren,[4] Spiegelman[5] and L'Héritier and co-workers[6, 7, 8] has focused attention on the rôle of the cytoplasm in the determination and inheritance of characters. The present paper deals with the same subject from a new point of view and using new techniques. As a consequence it has been possible to estimate the number of particles of a particular kind of cytoplasmic factor in *Paramecium aurelia* and to study the rate at which the particles increase while the paramecia themselves are reproducing by fission at a different rate. These studies turned out not only to be of interest in themselves, but also to bring out certain

remarkable facts concerning the inheritance of the character determined by the cytoplasmic factor. By taking advantage of the differential rate of increase of the particles of the cytoplasmic factor in relation to the rate of fission of the paramecia, it became possible, on the one hand, to free the paramecia from the particles and so produce strains permanently lacking them and the character they control; and, on the other hand, to induce cultures which had lost the character because of considerable reduction (but not total loss) of their particle number, to reacquire the normal number of cytoplasmic factor particles and the character associated with them. Finally a technique was devised which demonstrated that a single cytoplasmic factor particle in an animal is sufficient to give rise to the normal number of particles.

Our knowledge of cytoplasmic factors in *P. aurelia* began with Sonneborn's[1] investigation of the determination and inheritance of the character known as "killer." Organisms of this sort alter the fluid in which they live so as to make it poisonous to non-killer or "sensitive" stocks. The full genetic analysis has been carried out only for killers of variety 4 of this species. This killer character depends upon the presence of a cytoplasmic factor termed "kappa" which is maintained and increased only in the proper genetic background, namely in the presence of a dominant gene, K. If K is replaced by its recessive allele, k, kappa disappears in the course of a few fissions and the resulting clone lacks the killer character; in fact, it becomes sensitive to the action of killers. If the gene K is reintroduced into such a sensitive, the killer character does not reappear. Gene K cannot initiate the production of kappa, it can only control the maintenance and increase of kappa when some is already present. The killer character can be restored to sensitives with gene K by reintroducing some cytoplasm from a killer during conjugation, and kappa and the restored character are thereafter maintained permanently during reproduction.

Four killer stocks of *P. aurelia* (stocks *G*, *H*, 36 and 50) have been employed in this study. They all belong to variety 2, a member of the same group of varieties as the variety 4 reported on by Sonneborn. (For an account of the varieties of *P. aurelia* see Sonneborn and Dippell.[9]) Variety 2 is sexually isolated from the other varieties. Each of these four killers and the variety 4 killers differ in the manner in which they affect sensitive animals. The action of the variety 4 killers and certain of the variety 2 killers has been described by Sonneborn.[1, 10]

The determination and inheritance of these killer characters in variety 2 has not been reported except for Sonneborn's[1] remark that the observations thus far made agree with the findings in variety 4 and indicate the presence of cytoplasmic factors. This remark (Sonneborn, personal communication) is based simply on the observation that in the F_1 from a cross

of stock *G* by stock *H*, the two members of each conjugant pair produced clones with killer characters like the parent from which they derived their cytoplasm. This result is comparable to Sonneborn's[1] results in crosses of variety 4 killers to sensitives, in which the killer conjugant produced a killer clone, the sensitive conjugant a sensitive clone. In view of what is known further in variety 4, it therefore seems likely that cytoplasmic factors are also involved in the determination of the killer characters in variety 2. The word "kappa" will be used here as a general term to refer also to the various cytoplasmic factors associated with killing in variety 2. It is not meant to imply that these factors are identical in the various killer stocks.

The Control of Killing in Variety 2.—Soon after this study was begun a fundamental difference between the variety 2 and variety 4 killers became apparent. Killer stocks in variety 2, in striking contrast to those in variety 4, commonly give rise to non-killer, sensitive progeny. An investigation of the factors responsible for this production of sensitives revealed the following: (1) When animals are fed only a small amount of food so that a slow rate of fission is maintained (one-half fission per day, for example) all stocks remain pure killer indefinitely. On the other hand, when more food is made available and the rate of fission is increased, cultures of all four killer stocks lose their ability to kill and then become sensitive. Loss of killing is accomplished in 6 to 8 fissions at 3 fissions per day. The maximum rate of fission at which the stocks can grow and remain pure killer is apparently greatest for stock *G*. (2) Non-killer and sensitive cultures produced in this manner will revert back to killer after a few days when their growth is slowed or stopped, unless the fast growth has been maintained beyond a certain critical point. (3) The longer and the faster the rapid fissions are maintained, the longer it takes the animals in the cultures to revert to strong killers. (4) If rapid fissions are maintained for long enough, killing will never return. There seems to be but one reasonable explanation for these four facts. The hereditary basis for killing, kappa, fails to keep pace with the rate of growth of the animal; the amount per animal is progressively reduced in quantity by successive fissions; and finally animals are produced which have none at all.

Estimation of the Number of Particles of Kappa and Their Rate of Increase.—It should be possible to measure how long and how fast a strong killer has to be grown in order to rid it of kappa, and then to use this information to estimate the original number of particles and their rate of increase. An experiment designed to supply this information was started by isolating 192 of the progeny of a single original killer of stock *G* which had undergone eight rapid fissions at 27°C. Every time each isolation underwent 4 or 5 more fissions, one animal was transferred from each culture and a new set of 192 isolations was made. The remaining animals in each

of the 192 discard cultures left from each transfer continued for about 4 more rapid fissions when growth was suppressed to allow animals capable of reverting to killer to do so. It was then determined what proportion of each set of 192 discard cultures contained no killers. The proportion obtained for each set represents the proportion of isolated individuals which were incapable of producing killer progeny. If one assumes that one particle is sufficient to enable an animal to revert to killer and that particles are neither lost nor destroyed, then this proportion of animals producing no killer progeny is also the proportion of animals with no particles. Evidence in support of these assumptions is given in the next experiment. The data showed that after the original killer had undergone 12 fissions (in 3.6 days) the proportion of the 192 animals producing no killer progeny was 0.01; after 16 fissions (in 4.7 days), the proportion was 0.245; after 21 fissions (6.2 days), 0.74; after 26 (7.7 days), 0.93; after 31 (9.2 days), 0.97; and after 36 (10.7 days), 0.99.

If the distribution of the number of particles among the animals in the culture at any time is random, then the Poisson distribution can be used to determine the mean number of particles per animal from the observed proportion of animals with no particles. This can be done by making use of the first term of the Poisson series which relates the number of individuals with no particles (P_0) to the mean number of particles per animal (m) by the equation $P_0 = e^{-m}$ (where e is the base of natural logarithms). The applicability of the Poisson distribution, however, depends upon the condition that kappa is distributed randomly as discrete particles. Evidence that this is the case will appear subsequently. To Prof. H. J. Muller of Indiana University the author is indebted for pointing out that a correction must be introduced into the Poisson series to account for the slight nonrandomness introduced by the increase in number of the particles as the animals increase by fission. For the present, we will neglect this correction, and later on consider its magnitude. After 16 fissions, when P_0 was 0.245, it can be calculated, using the relation $P_0 = e^{-m}$, that m was 1.4; and after 21 fissions, when P_0 was 0.74, m was 0.30. But in going from 16 fissions to 21 fissions each animal produced 32 animals and its mean particle number of 1.4 must have produced $0.30 \times 32 = 9.6$ particles. If 1.4 particles produced 9.6 particles, then 1 particle produced $9.6/1.4 = 6.9$ particles. So the rate of increase of the particles may be expressed by saying that one particle produces 6.9 particles while the animals are going through 5 fissions in 1.5 days. Or it can be converted and expressed as 1.9 duplications per day for the particles while the animals are undergoing 3.3 fissions per day. This rate can now be used to predict the mean number of particles and also P_0 for any other fission, assuming the rate is constant. For example, the mean can be calculated to be 0.065 after the 26th fission, and this leads to a value of 0.94 for P_0 as compared

with an observed value of 0.93. All predicted values give good agreement, indicating that the assumption of the constancy of the rate is valid and also supporting the assumption of random distribution of discrete particles of kappa. If one assumes that the rate of 1.9 duplications per day has been constant during all the fissions back to the start of the experiment, the number of particles in the original killer (mean number after 0 fissions) can be computed in a similar manner. This number varies very slightly with the particular method used to make the calculation, but good agreement is obtained with a number of 180 particles. It is considered likely that the rate is probably somewhat less than 1.9 for the first few fissions, but it is thought that the error introduced into the estimate of 180 particles is probably small.

It was indicated above that an error was introduced into the calculations by the fact that the Poisson distribution does not fit the situation exactly. An approach has been made to this problem by a purely empirical method. A "pencil and paper" trial was run starting with a hypothetical animal with 180 particles. The particles were divided at random (use was made of Tippett's[11] "random sampling numbers") into two groups corresponding to a fission of the animal, and this was repeated for each fission. After 12, 16, 21, etc., fissions the number with no particles was compared with the experimental data. It was also necessary, of course, to increase the number of particles between each fission to simulate the calculated rate of 6.9 particles from 1, each 5 fissions (or 2.8 duplications for 5 fissions or 0.56 duplications for 1 fission). Actually, agreement was not good when a rate of 1.9 duplications per day and a particle number of 180 was used, due to the fact that these values were calculated on the basis of the Poisson distribution which is not strictly applicable. Different trials, however, showed excellent agreement if the rate was taken as 1.9 and the particle number as 256. These last values are thus considered to be the best solution to the problem.

Demonstration That a Single Particle of Kappa Is Sufficient to Enable an Animal to Revert to Killer.—An experiment was designed to test the assumption that one particle is all that is necessary to enable an animal to revert to killer under conditions of arrested growth. A number of rapidly dividing animals were originally isolated into separate containers and allowed to go through various numbers of fissions; all of the progeny of each original animal were kept in the container to form a culture. If the assumption is correct and no appreciable number of particles is lost or destroyed, then we would expect that any original animal containing a particle would give rise in the culture derived from it to at least some animals capable of reversion to killer. So that if, for example, 10% of the original animals had no particles, then 10% of the cultures would have no particles and hence no animals capable of reversion. Furthermore, this percentage

should be independent of the number of rapid fissions each original animal went through after isolation in the container. On the other hand, if more than one particle is necessary, then the percentage of the original animals giving rise to exclusively permanent sensitive progeny should increase as successive fissions cause the number of particles in *all* the progeny to drop below the number necessary for reversion.

The critical experiment was done starting originally with animals of stock *G* whose number of particles had been reduced by a series of rapid fissions. (These animals were obtained from a single killer which was allowed to go through 16 fissions at a rate of 3.3 fissions per day.) A number of these original animals were then removed individually to separate containers and allowed to undergo different numbers of rapid fissions at a rate of 3 fissions per day; then growth was stopped and it was determined whether *any* of the progeny of each of the original animals reverted to killer. It was found that 10.9% (14 out of 128) of the original animals after 8 rapid fissions gave rise to cultures of exclusively permanent sensitive progeny. And 10.2% (13 out of 127) of the original animals after 15 rapid fissions gave rise to exclusively permanent sensitive progeny. The fact that the percentage was not greater following 15 fissions than following 8 fissions is in agreement with the assumption that one particle is sufficient for reversion.

Summary and Discussion.—It has been shown that the cytoplasmic factor responsible for the killing phenotype in stocks of variety 2 of *P. aurelia* cannot increase as fast as the animals can be grown. By taking advantage of this differential growth rate it has been possible to reduce the number of particles of the cytoplasmic factor until the ability to kill is lost, and even to free animals of particles completely. Likewise, it has been established that animals which have been reduced to only a single particle will regain their normal number and become killers again when the fissions are slowed or stopped entirely. A study of the time and fission rate required to free animals of the particles has made it possible to estimate the number of particles as approximately 256 in one particular individual and also to calculate that the duplication rate of the particles was about 1.9 times per day while the paramecia were dividing 3.3 times per day.

It has been noted that the variety 4 killers never lose their killing power no matter how fast they are grown, as was pointed out by Sonneborn.[2] This is an important difference between the two varieties.

Sonneborn[2, 3] has discussed the possibility that the cytoplasmic factor, kappa, is bound to the gene *K* in the macronucleus where it is reproduced as a part of the gene. He has also considered that it can only pass from the nucleus into the cytoplasm at nuclear reorganization when the macronucleus breaks down. The reduction in number of particles by rapid growth described here for variety 2 is not in agreement with this view as

stated for variety 4, for in variety 2 the particles can be removed from the animals without the occurrence of macronuclear breakdown. In order to make this hypothesis fit the variety 2 situation the assumption would have to be made that in variety 2, unlike variety 4, the particles readily dissociate from the macronucleus and pass into the cytoplasm during vegetative reproduction.

L'Héritier and Tessier[6, 7] have reported on a "genoid" or cytoplasmic factor determining sensitivity to carbon dioxide in Drosophila. L'Héritier and Sigot[8] have shown that when small amounts of the cytoplasmic factor are introduced along with the sperm into the egg at fertilization, the inhibitory effect of extreme temperature reduces the rate of increase of the cytoplasmic factor to less than the division rate of the embryonic cells. They have shown that this results in the production of many cells lacking the cytoplasmic factor. This situation is remarkably similar to the reduction of the number of particles of the cytoplasmic factor in the variety 2 killers which was also accomplished by differential growth rates.

The behavior of the cytoplasmic factor described here calls attention to the significant fact that self reproducing cytoplasmic components may not increase at a rate strictly correlated with the growth rate of the cell. They may increase faster or slower, depending upon conditions. This cannot only cause a great variation in the amount of these components and their associated effects, but it can even result in their complete and permanent loss.

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